PREVENTION AND CONTROL OF GASTROENTERITIS IN CALIFORNIA LONG-TERM CARE FACILITIES

California Department of Health Services
Division of Communicable Disease Control
In Conjunction with Licensing and Certification

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The California Department of Health Services, Division of Communicable Disease Control, developed these recommendations in consultation with the Licensing and Certification Program, using information from the Centers for Disease Control and Prevention. This information is intended to be advisory only and was developed to assist facility infection control committees in the development of a rational approach to the prevention and control of gastroenteritis in long-term health care facilities.

Introduction

Gastroenteritis (various combinations of diarrhea, nausea, vomiting, and abdominal pain) is a relatively common condition in long-term care facilities (LTCFs). Age, underlying medical illnesses, alteration of gastrointestinal tract flora due to antimicrobial therapy, decreased gastrointestinal tract motility due to narcotic administration, and fecal incontinence contribute to increased susceptibility to enteric pathogens. The prevalence of diarrhea (percentage of residents with diarrhea at any given time) in nursing homes ranges between 0.5 and 1.5%.[1] Gastroenteritis accounted for approximately 5-15% of all infections in nursing homes. The incidence of acute gastroenteritis has been between 0.1 and 1.0 episode per 1000 resident-care days.[1]

Although most cases of infectious gastroenteritis in LTCFs are probably sporadic, outbreaks are not uncommon. In outbreaks of infectious gastroenteritis in LTCFs, attack rates (the percentage of residents who become ill) are high. It is not unusual for ¼ to ½ of residents in a facility to be affected. Outbreaks also tend to be prolonged, frequently lasting 10 or more days, when secondary cases (infections acquired from those who initially became ill) occur.

Infectious gastroenteritis often results in more severe illness in LTCF residents than in otherwise healthy young individuals. Dehydration, worsening of underlying disease, and sepsis increase the risk for severe illness and death in LTCF residents. One or more LTCF residents will be hospitalized in approximately 1 in 4 outbreaks. The case-fatality rate (percentage of those who become ill that die) in foodborne disease outbreaks is 10-fold higher in nursing residents than in the general public.[2]

The pathogens responsible for sporadic infectious gastroenteritis in LTCFs have not been well characterized. This is due in part to the difficulty in the diagnosis of gastroenteritis and the limited availability of laboratory tests in many facilities. Infections of toxin-producing *Clostridium difficile* probably account for much of the sporadic infectious gastroenteritis in LTCFs. This organism is resistant to most antibiotics and is associated with antibiotic use. It produces spores, which persist in the environment, and thus is a challenge for infection control measures (see, separately, "Prevention and Control of *Clostridium difficile* Disease in California Long-term Care Facilities").

The agents responsible for outbreaks in LTCFs are identified more often, although patients who are unable to provide symptoms or food consumption histories often compromise investigations. Information regarding the pathogens most commonly identified in LTCF outbreaks or that may be responsible for LTCF outbreaks is summarized in Table 1.

Table 1. Pathogens that may be involved in outbreaks of gastroenteritis in LTCFs in US

Causative Agent	Selected symptoms			Incubation period	Duration of illness	Mode of transmission	Characteristic foods
	Vomiting	Diarrhea	Fever				
Viruses							
Small, round, structured viruses	common	loose, watery, non-bloody	rare or mild	18-48 hours	12-48 hours	person-to-person, food, water	any food contaminated by food handler; shellfish
Bacteria							
Staphylococcus aureus	common	occasional	rare	1-6 hours	<24 hours	food	high protein, moist, often handled foods
Campylobacter jejuni	variable	often bloody	common	2-7 days	Usually <10 days	food, water, pets	poultry, raw milk
Salmonella	occasional	loose, watery, occasionally bloody	common	8-48 hours	3-5 days	food, water, pets	poultry, eggs, meat, sprouts, melons
Shigella	rare	often bloody	common	1-7 days	4-7 days (less if treated)	food, water, person-to-person	foods contaminated by food handler
Enterohemorrhagic *E. coli (e.g., E. coli* O157:H7)	common	first watery, then grossly bloody	rare or mild	3-5 days	1-12 days	food, person-to-person	hamburger, raw apple juice, raw milk
Yersinia enterocolitica	occasional	occasionally bloody	common	2-7 days	1 day-3 weeks	food, water, person-to-person	
Parasites							
Cryptosporidium	none	profuse, watery	occasional	1-2 weeks	4 days – weeks	food, water, pets, person-to-person	
Cyclospora	none	watery	rare	1 week	Few days to weeks	food, water	raspberries (imported)
Giardia lamblia	none	loose, pale, greasy stools	rare	5-25 days	1-2 weeks to months	food, water, person-to-person	any food contaminated by food handler

(ADAPTED FROM REFERENCE 8)

Salmonella is the most common pathogen identified in foodborne outbreaks and accounts for a disproportionate number of deaths. In the U.S. in 1975-1987, *Salmonella* accounted for 52% of foodborne disease outbreaks in nursing homes with a known cause, and 81% of the deaths in these oubreaks.[2] *Salmonella* outbreaks are consistently associated with high morbidity and mortality, with case-fatality rates of 2-9%. *Salmonella enteritidis* outbreaks accounted for 45% of the *Salmonella*-associated deaths since 1981. The implicated food vehicles in *S. enteriditis* outbreaks have typically involved raw or undercooked eggs. One outbreak in 1998 in a California nursing facility resulted in 6 bloodstream infections and one death in residents who requested either undercooked or scrambled eggs. A second resulted from using a blender to mix raw shell eggs for breakfast omelets and then to prepare pureed foods, causing illness in 15 residents and 2 staff. Illness from *Staphylococcal aureus* toxin is second only to *Salmonella* as an identified cause of foodborne disease outbreaks. In the U.S. in 1975-1987, Staphylococcal disease accounted for 23% of nursing home foodborne outbreaks with a known cause.

Viruses are rarely reported as causes of foodborne disease outbreaks in LTCFs. Viral gastroenteritis is difficult to diagnose, so the viral foodborne outbreaks that do occur go unrecognized. However, viral gastroenteritis is more often transmitted directly from person-to-person rather than by food. However, the initial case may have been acquired from food contaminated by a food handler or healthcare worker. This mode of transmission is characterized by a course that stretches over time, often many weeks, in contrast to foodborne outbreaks, in which cases are clustered together in time. Although viral gastroenteritis is usually a mild disease, death occasionally occurs in frail LTCF residents. Due to the prolonged nature of outbreaks of viral gastroenteritis and the need to cohort residents and staff to control them, they are often costly and disruptive, even when the degree of illness is mild.

Prevention and control of gastroenteritis depends upon the following infection control elements: surveillance, general infection control measures, and identification and control of outbreaks. The principal function of surveillance is to monitor and detect increases in the incidence of endemic disease. Routine surveillance may be of use in the detection of outbreaks, particularly those in which the initial attack rate (percentage of residents who are ill) is low.

General infection control measures will limit endemic infectious gastroenteritis by limiting the spread of organisms such as *C. difficile* and will also be effective in limiting the impact of outbreaks when there is prompt isolation of residents and work restriction of employees with acute gastroenteritis. Key to this strategy is recognition of the importance of the fecal-oral route for person-to-person transmission of most enteric pathogens and implementation of appropriate measures to break this chain of transmission.

Clusters and outbreaks of gastroenteritis may be detected through routine surveillance as well as by diligent staff observation and reporting. Clusters of gastrointestinal disease occur commonly, but may not be specifically identified or reported as outbreaks. Management includes epidemiologic investigation, identification of the etiologic agent and source, control measures including elimination or treatment of the source, and staff education to limit transmission and to prevent future outbreaks. Until an etiologic agent is identified, secondary transmission can be limited through strict adherence to contact precautions for affected residents, including handwashing before and after seeing each patient and wearing gloves and gowns when cleaning patients and disposing of stool. In outbreaks where many patients are ill, it may be advisable to separate infected and uninfected residents by area or by room and to assign nursing personnel to work separately with these groups.

The data in Table 1 indicate the need for a number of different prevention strategies depending upon the etiologic agent. Acquisition of viral gastroenteritis in the community by staff is inevitable and beyond the control of the facility. However, exclusion of ill employees may prevent its introduction into the facility, and separation of ill residents from the well will limit its spread after introduction.

Outbreaks of *Salmonella* and other foodborne agents can be prevented by thorough cooking of all meat, poultry, and eggs, by rapid chilling to refrigeration temperature of cooked foods not "meant" or "intended" to be used immediately, and by preventing cross-contamination of cooked food with raw food. Specifications for these and other aspects of food preparation are available in the Food Code, published by the Food and Drug Administration. The Food Code is a reference that guides retail outlets such as restaurants and grocery stores and institutions such as nursing homes on how to prevent foodborne illness. Local, state and federal regulators, including the California Department of Health Services, use the FDA *Food Code* as a model to help develop or update their own food safety rules and to be consistent with national food regulatory policy. The Food Code is updated every two years. It can be obtained on-line at http://vm.cfsan.fda.gov/~dms/foodcode.html, or in hard copy or on computer disk from the National Technical Information Service at 5285 Port Royal Road, Springfield, VA 22161; Phone 703-605-6000 or 1-800-553-NTIS (6847).

Salmonella enteritidis outbreaks can be prevented through the use of pasteurized egg products as substitutes for raw shell eggs. Additional foods that should never be served to susceptible populations such as nursing home residents include raw seed sprouts (e.g., alfalfa, clover, bean), which have caused outbreaks of *Salmonella* and *Escherichia coli* O157:H7 infections in many countries. Raw animal foods such as raw fish, raw-marinated fish, raw shellfish, and steak tartare, or partially cooked animal food such as lightly cooked fish, rare meat, soft-cooked eggs that are made from raw shell eggs, and meringue should never be served to susceptible persons. Prepackaged juice or a prepackaged beverage containing juice that have not been specifically processed to prevent, reduce, or eliminate the presence of pathogens, such as pasteurization, also should not be served. Outbreaks of *Escherichia coli* O157:H7 infections have occurred as a result of the contamination of apples used in unpasteurized apple juice or cider.

SURVEILLANCE

Surveillance is the cornerstone of all infection prevention and control programs. All facilities should establish and maintain a program of surveillance for infectious gastrointestinal disease. At a minimum, a baseline rate for diarrhea should be established. Once a baseline rate is determined, it would be more readily apparent that a sizeable increase in new cases suggests the occurrence of an outbreak, for which special interventions may be necessary.

Surveillance should include the following components:

1. Clinical definitions for identification of individual cases of gastroenteritis.
   A proposed definition of gastroenteritis for surveillance in LTCFs[1] is one of the following: (1) two or more loose or watery stools above what is normal within a 24-hour period, (2) two or more episodes of vomiting in a 24-hour period, or (3) a stool culture positive for *Salmonella*, *Shigella*, *E. coli* O157:H7, or *Campylobacter* or a toxin assay positive for *C. difficile* toxin and one symptom or sign of gastrointestinal infection (nausea, vomiting, abdominal pain or tenderness, or diarrhea).

2. Criteria that define when specimens and laboratory tests should be obtained (see "Prevention and Control of *Clostridium difficile* Disease in California Long-term Care Facilities").

3. A separate database (line listing or logbook) for each gastroenteritis case. Include the following information: (1) name, (2) age, (3) sex, (4) location in institution, (5) laboratory test and results, (6) treatment and results.

4. Calculate rate (cases divided by resident-days or by residents per month or year if resident-days unavailable) for diarrhea and compare to baseline prior to each infection control committee meeting and any time there is suspicion of an increased rate.

MANAGEMENT OF OUTBREAKS

As for all outbreaks, the primary goals of outbreak management are to control and prevent further disease and to identify factors that contributed to the outbreak. The steps are to: (1) confirm that an outbreak exists; (2) institute initial control measures; (3) find additional cases; (4) identify the etiologic agent; (4) characterize the cases by person, place, and time; (5) make an initial hypothesis ("best guess") to explain the outbreak; (6) implement study to identify risk factors; (7) evaluate control measures; and (8) communicate findings.[3]

Confirm Outbreak

First establish a case definition (for example, diarrhea in a resident), based on initial assessment of the illnesses. The definition may be changed later based on additional information (for example, diarrhea and/or vomiting in a resident or employee with onset in the previous 7 days). Count the number of known cases, calculate a rate (number of cases divided by total number of residents) and compare that to the usual or baseline rate, using surveillance data if available**.**

Report Outbreak

If an outbreak is suspected, report this immediately to <u>both</u> the local (usually county) health department <u>and</u> to the Licensing and Certification district office. Reporting to the local health officer is required by California Code of Regulations (CCR) Section 72537 and reporting to Licensing and Certification ("the Department" in the regulations) is required by CCR 72541, for SNFs. Individual cases illness due to *Salmonella*, *Shigella*, *Staphylococcus aureus* (gastroenteritis only), *Bacillus cereus*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *E. coli* O157:H7 must be reported to both agencies even in the absence of an outbreak.

Institute Initial Control Measures

Initial control measures should be instituted based on the magnitude and nature of the problem. In most cases these will include measures to separate the affected from the unaffected. Residents who are not ill but may have been exposed to a source may be incubating an infection and already be contagious. Consideration should be given to separating those exposed from those who are well and not exposed. In all situations, ill employees should be excluded from patient care. Any specimens that might require further evaluation (food items, previously submitted laboratory specimens) should be secured and stored appropriately (see Appendix B).

Find Additional Cases

The case definition established previously should be refined, if necessary, with specific criteria. Then potential cases should be characterized by signs, symptoms, and laboratory findings. Those ill persons who meet the case definition are entered in a line listing. A record of those who are ill but do not meet the case definition may be helpful in evaluating the adequacy of the case definition later. A system must be established to ensure prompt reporting of all new potential cases. A search of records for previously occurring cases that might have been undetected or unreported should be conducted. Early institution of a specific data collection form, with space for future entry of exposure information, is helpful. An example is provided in Appendix A. Selected items can be abstracted on the line listing.

Identify Etiologic Agent

Early identification of the responsible agent is a key step in controlling outbreaks. In most cases, consultation with the local health department will be necessary to determine what specimens should be collected and how they should be stored and transferred to a laboratory for evaluation. Information relevant to outbreaks according to specific pathogens is summarized in Table 1. Food, water, and ice should be submitted for testing, by prior arrangement with the appropriate laboratory, only if they are epidemiologically implicated as the source of the outbreak. Specific recommendations for the collection of laboratory specimens in the investigation of outbreaks of gastroenteritis are included in Appendix A.

Viruses

Most outbreaks of viral gastroenteritis in LTCFs are due to a group called small, round, structured viruses (SRSVs) or caliciviruses viruses, which include Norwalk and related viruses. These viruses cause illness with similar characteristics. Persons with viral gastroenteritis may have any combination of nausea and vomiting, diarrhea, and low-grade or no fever. Abdominal cramps, chills, and weakness may be present. When viral gastroenteritis occurs in winter it is often referred to as “intestinal influenza”. Illness will begin between 18 and 48 hours following exposure, such as to an ill resident or employee. Unless complicated by underlying illness, age, or dehydration, the illness will generally be mild and of short duration.

These viruses are spread when material contaminated by feces or vomitus from an infected person is ingested. However, contaminated shellfish (e.g., oysters, clams), items unlikely to be served in LTCFs, have been responsible for foodborne outbreaks. Any infected foodhandler (including food servers) can contaminate any food by vomitus or fecal contamination. Most patients shed these viruses in the greatest amounts during the acute phase of illness, but immunocompromised patients may shed viruses for months. Airborne transmission may occur through aerosolization during forceful vomiting. Direct transmission from animals has not been reported.

The traditional method of laboratory identification of viral gastroenteritis agents has been immune electron microscopy of stool, which requires specialized laboratory equipment of limited availability, large quantities of fresh (unfrozen, collected during the first 48 hours of illness) diarrheal stool. Low sensitivity of this technique means that negative results are of little significance. Newer techniques such as polymerase chain reaction (PCR) are faster and more sensitive but are available in only a few specialized laboratories. Serologic diagnosis is possible but must wait for the availability of convalescent sera (typically collected about 4 weeks after onset of illness).

Bacteria

Many different bacteria cause outbreaks of gastroenteritis (See Table 1). Although *E. coli* has long been known to cause diarrhea, the recent identification of new strains suggests that this pathogen may be a more important cause of diarrheal illness than was previously recognized. Enterohemorrhagic *E. coli* (*E. coli* 0157:H7 is the most common found in the U.S.) was first recognized as a cause of human illness during an outbreak of hemorrhagic colitis in 1982. The young and the old are susceptible to a complication of this hemorrhagic colitis called hemolytic uremic syndrome (HUS). Outbreaks of diarrhea and HUS have been caused by ingestion of undercooked ground beef, raw milk, raw apple juice, lettuce, and sprouts. Identification of these organisms can require specialized laboratory techniques such as serotyping, and specific toxin-detection tests that may not be routinely available in clinical laboratories.

Parasites

Cryptosporidium, *Cyclospora*, and *Giardia lamblia* are parasitic diarrheal pathogens with a worldwide distribution. All are known to have caused outbreaks of diarrhea in the United States, however, parasites rarely are responsible for disease in LTCFs.

With the use of new concentrating and staining techniques, investigators have recently shown *Cryptosporidium* to be an important pathogen in the United States. *Cryptosporidium* has been associated with outbreaks of diarrhea in child daycare centers. Outbreaks of diarrhea from *Cyclospora* in the United States have been principally associated with raspberries imported from Guatemala.

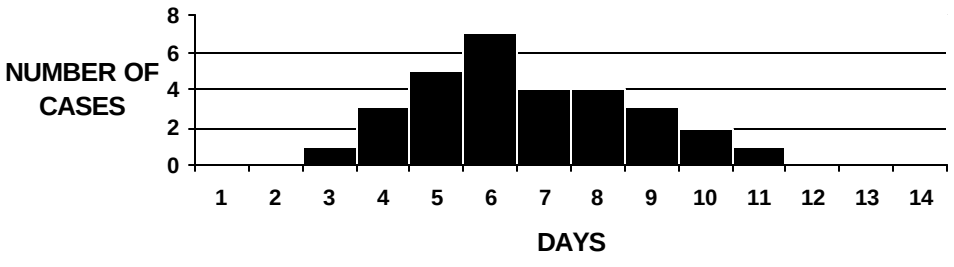
In general, the incubation period, duration of illness, and period of pathogen excretion are usually longer with diarrheal infections caused by parasites than by viruses or bacteria. For most patients, the incubation period for *Cryptosporidium*-related illness is approximately 5 days. Symptoms include transient diarrhea with profuse watery stools, abdominal cramps, and occasional fever and nausea with or without vomiting. The duration of illness is usually 3 days to 3 weeks, but illness has been documented to last for greater than 1 month. The staining techniques required for laboratory detection of parasites means that the tests must be specifically ordered.

Characterize Cases

Time

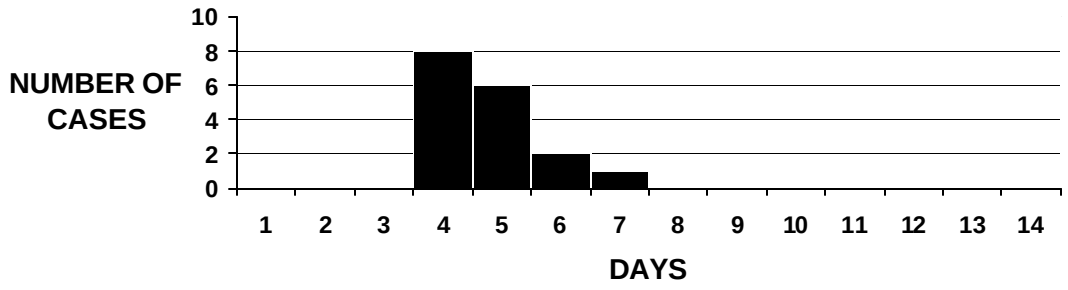
Establish the exact period of the outbreak by going back to the first case or first indication of outbreak activity. Record the date of onset of illness for cases and prepare an epidemic curve. An epidemic curve is a simple bar histogram plot of cases, one case per line, plotted against time, one day per bar (Figure 1).

Figure 1. Epidemic curve, example



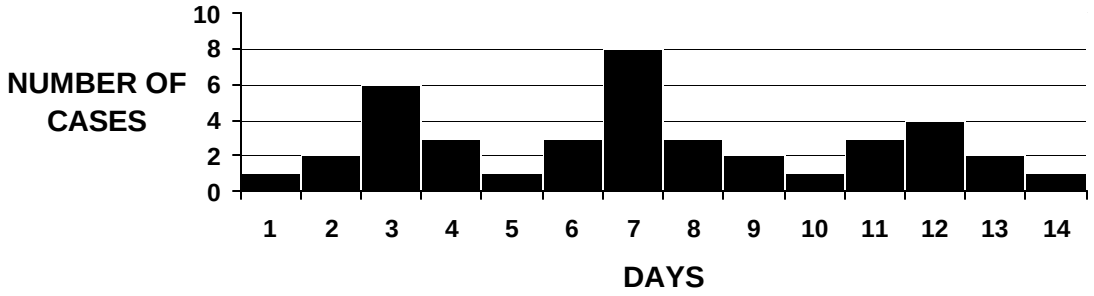
Clustering of cases at one time suggests a common source (Figure 2).

Figure 2. Epidemic curve, common source



Lack of clustering or successive multiple waves of cases suggests a propagated (person-to-person) outbreak (Figure 3).

Figure 3. Epidemic curve, person-to-person transmission



If the diagnosis (etiologic agent) has been identified or hypothesized, estimate the probable period or periods of exposure by plotting back the length of the incubation period from the time of onset for cases.

Place

Identify cases according to location in the facility, usually by wing, ward, floor, or nursing station. The use of tables or maps showing the distribution of cases by place may show clustering of cases. Clustering may indicate risk factors (food, nursing or support staff) that merit further investigation.

Person

Patient characteristics (such as age, sex, age, underlying disease, and diet) should be recorded on data collection forms. Interviews with residents and staff, including food handlers, should be conducted as soon as possible. The cognitive status of residents must be assessed to determine the utility of interviews, and chart review may need to be used, instead. With the exception of an unusually large outbreak in a large facility, data should be collected, by interview if possible, for all that were exposed, whether ill or not. For most outbreaks, investigations that require detailed interviews and analysis of data will be conducted by staff from outside the facility, including staff from local health departments, licensing and certification, and/or consultants to the facility. Information to assist in such investigation and analysis are included in Appendix C.

Formulate hypothesis

Based on the information collected and analyzed, a hypothesis (best guess) on the reservoir, source, and mode of transmission of the outbreak should be formulated. If a formal investigation is conducted (Appendix C), the analyses from this will be used to formulate the hypothesis. If not, information collected by facility infection control staff can be used. The hypothesis should explain the majority of cases. Frequently there will be cases occurring during the outbreak that are not explained by the hypothesis. These may be sporadic (unrelated to the outbreak) cases of the same disease, cases of a different disease with similar symptoms, or the same disease with a different source or mode of transmission.

Institute and Evaluate Control Measures

Initial control measures should have been instituted previously. Continue to monitor for further cases. If the control measures fit the hypothesis (the measures are directed to those factors identified through the investigation as responsible for transmission) and appear to be effective (cases cease to occur or return to endemic level), then they do not need to be changed. If cases continue to occur above the previous endemic level, reevaluate control measures in respect to the factors that might be responsible for transmission. Always attempt to use this opportunity to review and correct other facility practices related to the current situation, which may contribute to a similar outbreak in the future.

Communicate Findings

A written report should be prepared once the outbreak is under control. This will be facilitated if written documentation was occurring during the outbreak and its investigation. The report should include an introduction (description of the problem and how it was first recognized), methods (case definitions, sources of data, study design), results (facts only), and discussion (interpretation of results, description of control measures, recommendations for future surveillance and control). Copies should be provided to appropriate facility staff, the local health department, and the Department of Health Services Licensing and Certification district office.

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APPENDIX A. PROCEDURES FOR COLLECTING AND STORING SPECIMENS

Adapted from Reference 8 (see page 11)

While culture for standard bacterial pathogens should usually be performed routinely, analyses for viruses, special bacterial pathogens, and parasites must be ordered specifically. Due to the special nature and expense in the collection, handling and analysis of specimens for these pathogens, these are usually ordered only when the clinical and epidemiologic nature of the outbreak raises suspicion of their presence. Because appropriate specimen-collection methods for viral, bacterial, and parasitic agents differ, the sections below are divided into by agent. Submit a list of specimens and a specimen submittal form for each specimen. Specimen submittal forms are available from your local health department or by calling the Viral and Rickettsial Disease Laboratory (510-540-2587) for suspected viral outbreaks or the Microbial Disease Laboratory (510-540-2442) for suspected bacterial or parasitic outbreaks.

NOTE: Certain specimens of food, water, or ice can be submitted for testing if they are epidemiologically implicated as the vehicle of the disease. However, because of the technical difficulties and the low yield associated with such studies, only send such specimens after staff in the appropriate laboratory have been contacted and approve. Refrigerate specimens for viral and parasitic diagnostic testing at 4° C, and freeze specimens for bacterial diagnostics at -70° C.

A. Stool (See Table A1)

1. General guidelines

a. Timing. Begin collecting stool specimens immediately after being notified of an outbreak, since delay may impede identification of the causative agent. To permit diagnosis of certain viral agents, **specimens must be collected during the first 48 hours of illness.** For bacterial pathogens, stool should be collected before antimicrobial agents are given (or, if necessary, 2-3 days after cessation of therapy).

b. Quantity. Collect diarrheal stool samples from **at least 10 ill persons** (assuming that at least that number are involved in the outbreak). For outbreaks thought to be of viral origin, collect large-volume (at least 8 oz) stool specimens.

2. Methods for collection and storage

a. Viruses. Place each diarrheal stool specimen, of as large a quantity as can be obtained, in a leak-proof, clean, dry container, and refrigerate at 4° C. Instructing patients to catch stool specimens in plastic kitchen wrap draped across the back half of the toilet under the toilet seat, or in large-mouthed containers or a clean, disinfected bed pan before pouring into a container, may facilitate collection of stool specimens. Do not freeze specimens if electron microscope examination is anticipated.

b. Bacteria. Collect bulk stool if possible and place in specimen container containing preservatives (obtained from local health department or other laboratory). Swabs of fresh stools or rectal swabs may be collected if bulk stools cannot be obtained. Place swabs in refrigerated (i.e., chilled 1-2 hours before use) Cary-Blair transport medium. Ensure that visible fecal material is present on each swab.

IMPORTANT: Refrigerate or freeze tubes after specimens are placed in them. Specimens should be sent and tested within 24 hours after collection. If specimens must be held longer than 48 hours, freeze them as soon as possible after they are collected. Storage in an ultra-low freezer (-70° C) is preferable. Storage in a home-type freezer, if thaw cycles can be avoided, is acceptable for short periods if necessary.

TABLE A1. General instructions for collection of stool specimens*

Instructions for collecting specimens	Type of agent to be tested for		
	Virus	**Bacterium**	**Parasite**
When to collect.	As soon as possible - no later than 48 hours after illness.	During period of active diarrhea (preferably as soon after onset of illness as possible).	Any time after onset of illness (preferably as soon after onset of illness as possible).
How much to collect.	As much stool sample from each of 10 ill persons as possible (at least 10 cc per person).	Fresh stool (or swabs with visible fresh fecal material if necessary) from each of 10 ill persons.	A fresh stool sample from each of 10 ill persons.
Method of collection.	Place fresh stool specimens (liquid preferable) unmixed with urine, in clean dry containers (e.g., urine specimen cups). Do not add any liquid or preservatives. Do not collect in Carey-Blair medium.	For bulk stool, place in container with preservative (obtained from laboratory). For swabs, place into Cary-Blair medium tube. Break off top portions of swab sticks and discard sticks.	Collect a bulk sample, unmixed with urine, in a clean container. Place a portion of each stool sample into 10% formalin and polyvinyl alcohol preservatives at a ratio of 1 part stool to 3 parts preservative. Mix well.
Storage of specimen after collection.	Immediately refrigerate at 4° C. DO NOT FREEZE if electron microscopy is anticipated.	Immediately refrigerate at 4° C if testing is to be done within 48 hours (should be within 24 hours); otherwise freeze samples at -70° C.	Store at room temperature or immediately refrigerate at 4° C. DO NOT FREEZE.
Transportation	Keep refrigerated. Place bagged and sealed specimens on ice or with frozen refrigerant pack in an insulated box. Send by overnight mail. DO NOT FREEZE	Refrigerate as directed for viral specimens. For frozen samples: place bagged and sealed on dry ice. Mail in insulated box by overnight mail.	Refrigerate as directed for viral specimens. For room-temperature samples: mail in waterproof containers. DO NOT FREEZE.

*Label each specimen container with a waterproof marker with patient's last name, first name and date collected. Put samples in sealed, waterproof containers (e.g., plastic bags). Batch collection and send by overnight mail, scheduled to arrive at destination on a weekday during business hours.

c. ***Parasites.*** Mix fresh bulk-stool specimens thoroughly with each of two preservatives, 10% formalin and polyvinyl alcohol (PVA) fixative, at a ratio of 1 part stool to 3 parts preservative. Preservatives are available in commercial kits. If there is a delay in obtaining the preservatives, refrigerate untreated stool specimens at 4° C (do not freeze) for up to 48 hours. Once preserved, the specimens can be stored and transported at room temperature or refrigerated. Do not freeze.

3. Transportation (specimen handling)

a. ***Refrigerated specimens.*** Enclose each specimen in a secure container to which has been affixed a waterproof label. Place this container in a waterproof bag with tissue, towels, or other blotting material to absorb any leakage. Batch specimen containers as long as this does result in delay beyond 24 hours, pack with ice or frozen refrigerant packs in an insulated box, and send by overnight mail scheduled to be delivered during business hours on a weekday.

b. ***Frozen specimens (for bacterial testing only).*** So that they remain frozen, ship frozen specimens on dry ice. Use enough dry ice to keep the specimen frozen until it is received at the laboratory that will process it (i.e., enough dry ice to fill one-third to one-half of the shipping container). Do not allow glass tubes to be in direct contact with dry ice. Place a layer of paper or other material between the tubes and the dry ice. To prevent excess exposure of specimens to carbon dioxide, tighten the screw caps on the Cary-Blair tubes and seal them with electrical tape or seal the specimens in a plastic bag within the container of dry ice.

B. Serum

1. General guidelines

a. ***Timing of collection of specimens.*** Submit two serum specimens (an acute-phase specimen and a convalescent-phase specimen) for each patient thought to have illness caused by viruses. Obtain the acute-phase serum specimen as close to the time of onset of illness as possible (at most, within 10 days after onset) and the convalescent-phase serum specimen 4-6 weeks after the onset of illness.

b. ***Source(s) of specimens.*** If possible, obtain paired serum specimens from the same 10 patients from whom stool samples were obtained. Ten paired serum specimens obtained from well persons can serve as control specimens.

2. Methods for collection and storage

Collect blood specimens from adults (15 ml) and from children (3 ml) in tubes that do not contain anticoagulants (usually red-top tubes). Centrifuge the blood and send only the serum for analysis. If no centrifuge is available, store the blood specimens in a refrigerator until a clot has formed. Then remove the serum and pipette it into an empty sterile tube (using a Pasteur pipette). Refrigerate the tubes of spun serum until they are shipped. Refrigerate, but do not freeze, tubes containing unspun serum.

3. Transportation

Ship serum specimens either refrigerated or frozen. If the clotting technique described above is used to obtain the serum, ship the specimens refrigerated so that they can be centrifuged before they are frozen. Specimens can be refrigerated by placing them in an insulated box with ice or frozen refrigerant packs. Frozen specimens can be kept frozen by shipping them on dry ice. Batch the specimens, and send by overnight mail that is scheduled to arrive at the laboratory during business hours on a weekday.

APPENDIX B. PROCEDURES FOR DETECTION OF PATHOGENS

Adapted from Reference 8 (see page 11)

A. Viruses

1. General guidelines

Some of the tests presently available are direct EM; immune EM; polyacrylamide gel electrophoresis; enzyme immune assays for rotavirus (groups A and B), enteric adenovirus, and astrovirus antigens; serologic enzyme immune assay for SRSVs; and polymerase chain reaction (PCR) for SRSVs and other viruses.

a. **Stool.** Many viruses--including rotaviruses, adenoviruses, astroviruses, caliciviruses including Norwalk virus, and other small, round structured viruses--can be detected with EM. Because the electron microscope scans a field containing a millionth of a milliliter of stool, the specimen must contain at least a million particles per milliliter for one particle of virus to be detected. In order to assure that samples contain the highest concentration of virus possible, specimens must be collected as close to the onset of illness as possible, generally within the first 48 hours.
Freezing may alter the morphologic characteristics of some viruses; therefore, samples should be kept refrigerated at 4° C and should not be frozen if electron microscopy examination is anticipated. Because most enteric viruses cannot be cultivated, reagents for diagnostic tests are limited. In fact, because human stool samples are the basic source of antigen and virus, the bulk collection of specimens is always encouraged. Proper collection and storage of large-volume specimens will facilitate electron microscope examination and allow for concentration methods that enhance the likelihood of successful testing.

b. Serum. Antibodies to viruses usually begin to rise the first 7-10 days after onset of illness, peak around the fourth week, and can begin to fall by the 6-8 weeks after onset (particularly for SRSVs). Acute-phase serum specimens should be collected in the first week of illness and convalescent-phase serum specimens from the third to the sixth week after onset of illness. Since many persons have preexisting antibodies to some of the viral agents, a single convalescent-phase serum sample is generally of little diagnostic value. At present, determination of the cause of outbreaks of viral gastroenteritis relies heavily on assays of serologic response. Paired serum specimens should therefore be submitted, with stool, for testing associated with all such outbreaks. A fourfold rise in specific antibody titer between acute-and convalescent-phase serum samples is accepted as diagnostic of recent infection.

B. Bacteria

2. General guidelines

Tests available for identification of organisms include isolation procedures for *Salmonella, Shigella, Campylobacter, Vibrio, Yersinia enterocolitica, Aeromonas hydrophilia, Plesiomonas shigelloides, Bacillus cereus, Staphylococcus aureus,* and *Escherichia coli,* as well as serodiagnostic assays for *Vibrio cholerae,* and *E. coli* 0157:H7. When no other pathogen is found, serotyping of *E. coli* recovered from ill persons and from appropriately chosen controls can identify previously unidentified *E.*

coli pathogens. Tests for Shiga-like toxin-producing E. coli are also available. Subtyping methods are available to determine the relatedness of enteric bacteria.

a. **Stool.** A pathogenic bacterium can be identified by isolating the organism in culture, by serotyping, or by identifying a characteristic marker for virulence. Fresh stool specimens should always be used when possible to ensure that fastidious organisms and toxins that decompose easily are detected before they degenerate. Expedient refrigeration of specimens is important because bacteria can easily be overgrown by competing organisms in stool specimens left at room temperature for greater than 4 hours. If testing must be delayed beyond 48 hours after the specimen is collected, the specimen should be frozen to retard the overgrowth of bacteria. Since most bacterial pathogens can be cultured from appropriately acquired rectal swab specimens, rectal swabs or swabs of fresh stools are preferred to bulk stool specimens. This method of collection also facilitates storage and transport.

b. **Serum.** Specific antibody testing may be possible for some bacterial enteric agents such as Shigella. As is true for viral infections, antibody titers to bacterial agents generally rise by the end of the first week after onset of illness and peak by 3-4 weeks. A fourfold rise in specific antibody titer is accepted as diagnostic of a recent infection.

C. Parasites

1. **General guidelines** Some special stains and other tests available for formalin-preserved specimens.
2.
   a. **Stool.** Parasites are usually detected and identified with microscopy of fresh or appropriately preserved stool specimen (although parasites can sometimes be grown on special media). Fresh specimens are needed for direct microscopy because trophozoites are fragile and may not survive environmental stress. The PVA and formalin fixatives preserve the morphology of cysts and trophozoites in stool specimens for diagnostic testing.

   b. **Serum.** Unlike outbreaks of gastroenteritis due to bacteria or viruses, an outbreak of parasitic diarrheal illness can sometimes be identified with a convalescent-phase serum specimen only. However, it is sometimes necessary to have both acute and convalescent serum specimens to make a definitive diagnosis.

APPENDIX C. ANALYSIS OF OUTBREAK DATA

Food should be suspected as a source if cases appeared over a short time interval throughout a facility. If possible, all subjects should be interviewed or assessed for food exposures during the exposure period.[4] Menus should be obtained or recreated for the exposure period and each subject asked specifically whether available items were eaten. Data to be entered in a table includes specific food items, those who **ate** the specified food (split into those who were ill and those who were well), and those who did **not** the specified food (split into those who were ill and those who were well) (Tables C1 and C2).

If the entire population (the cohort) is available for study, then the search for implicated food items is done through a cohort study - in this case a retrospective (looking-back) cohort study (Table C1). If the outbreak population is large (over 100) and it is not possible to collect information from all, a random sample should be selected and information collected on symptoms and food exposure history. Rates of illness in those who did eat specific food items are calculated and compared with the rates of illness in those who did not eat those items. For each food item, attack rates in those who did or did not eat the item are calculated by dividing the number ill by the total number either exposed or unexposed to those foods. Implicated food items will generally have the highest attack rates. In the final column of the table, the attack rate for the eaters is divided by that of the noneaters for each item, generating Relative Risks for each. If the relative risk is significantly greater than 1.0 (typically $\geq$3), there may be an association between food exposure and illness. It is important to calculate relative risks since high attack rates may be present for a food item that was consumed by a large percentage of residents, both ill and well. Attack rates are usually not 100% for implicated food items since: (1) the food may not be contaminated throughout; (2) susceptibility to the agent may vary; (3) dosage (the amount of the agent consumed) may vary; (4) food histories may be in error; (5) those who are ill but report no exposure may have had a coincidental, unrelated illness or may have been exposed to some other source (an ill person or other contaminated food item or utensil).

Table C1. Cohort (relative risk) calculations

Food Item	Ill	Well	Total	Attack Rate	Relative Risk
Eaten	a	b	a+b	a / (a+b)	$\frac{a / (a+b)}{c / (c+d)}$
Not eaten	c	d	c+d	c / (c+d)	

When the overall attack rate (the percentage of residents and/or staff that are ill) in an outbreak is small, or not all of the population is available for study, a slightly different design (called "case-control") is used. A table similar to that for a cohort study is constructed (Table C2), but the frequencies with which specific food items were selected (food preference rates) by those ill and those well are calculated. These are calculated by dividing the number of each who ate by those who did not eat each item. The ratios of food preference rates (rates of selection by cases divided by the rates in controls) results in Odds Ratios for each food item. If the odds ratio is significantly greater than 2.0 (and the confidence interval does not include 1.0), there may be an association between food exposure and illness.

Table C2. Case-control (odds ratio) calculations

Food Item	Ill	Well	Odds Ratio
Eaten	a	b	
Not eaten	c	d	
Food Preference Rate	$\frac{a}{c}$	$\frac{b}{d}$	$\frac{a/c}{b/d} = \frac{ad}{bc}$

Person-to-person or some other mode of transmission should be suspected when cases occur over a longer period of time with clustering by unit, floor, wing, or caregiver patterns. An investigation similar to that for food can be conducted, but exposures other than food (e.g., staff contact, attendance at some event, medication) are recorded instead of food. Collection of accurate data on exposures such as staff contact may be more difficult and time consuming than that for food. Attack rates for such exposures may be lower (for example, most contacts with an ill staff member will not result in illness) but still should generate elevated risk or odds ratios (for example, more ill residents will have had contact with an implicated staff member than did well residents). Investigations that may result in the implication of staff in the initiation or propagation of an outbreak must be conducted with sensitivity to the psychological and legal consequences. Information should always be communicated with the understanding that epidemiological "associations" and "implications" do not necessarily constitute "proof" of causality.